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Molecular characterization of Corynebacterium pseudotuberculosis isolated from sheep by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR)

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The phenogenetic and phylogenetic relationships among 7 identified strains of *C. pseudotuberculosis* isolated from sheep were checked by PCR specific primers demonstrated with random amplified polymorphic DNA – polymerase chain reaction (RAPD – PCR) by using five arbitrarily primers. *C pseudotuberculosis* isolates were distinguished according to the banding patterns of their amplified DNA on agarose gel. The variation can be used for diagnostic differentiation among *C. pseudotuberculosis* strains. Differences were observed in RAPD patterns between the 7 isolates and this may be due to the presence of novel serovars of this microorganism.

Corynebacterium pseudotuberculosis (C. pseudotuberculosis) is the causative organism of caseous lymphadenitis (CL) and has been isolated from sheep, goats, buffaloes and horses (Barakat *et al*., 1984; Yeruhan *et al*., 1997; Selim, 2001). Also the infection has been reported in humans (Peel *et al*., 1997). Caseous lymphadenitis is prevalent worldwide, but incidence is higher in areas where intensive husbandry is practiced (Literak *et al*., 1999). Sheep and goats industries worldwide suffer significant economic loss due to the culling of infected animals, carcass condemnation and decrease wool production (Pepin *et al*., 1994). The disease is characterized by the formation of encapsulated necrotic or caseous lesions that in sheep are located primarily in peripheral lymph nodes and lungs (Walker *et al*., 1994). *C. pseudotuberculosis* is a facultative intracellular pathogen that multiplies within macrophages. It is readily phagocytosed by non immune macrophages, and following phagocytosis with ovine (Hard, 1972) and caprine (Tashjian and Campbell, 1983) macrophages, phagosomes fuse with lysosomes but bacteria continue to multiply within phagolysosomes, leading to cell death, release of bacteria and ultimately a necrotic lesion (Walker *et al*., 1994). The pathogenic process employed by *C. pseudotuberculosis* in causing caseous lymphadenitis in sheep and goats is not well defined; at least two major virulence determinants have been identified. One of which is the toxic lipid cell wall, which may mediate bacterial resistance to killing by phagocytic cells (Cameron *et al*., 1998). The other identified virulence determinant is a sphingomyelin-degrading phospholypase D (PLD) exotoxin (Cameron *et al*., 1998). PLD is thought to mediate dissemination of the pathogen within the host by increasing local vascular permeability (Baty, 1986). *C. pseudotuberculosis* can be differentiated into genetically distinct isolates using procedures that identify difference in genetic composition of microbial population as random amplified polymorphic DNA (RAPD) (Welsh and McCelland, 1991). The arbitrary DNA-PCR has been used to characterize and differentiate bacterial strains and this method considered faster, relatively simpler and more economical than other genomic typing method (Elaichouni *et al*., 1994). When single primer was used in arbitrary primers PCR, discrete and limited portion of genome were amplified, producing a characteristic set of amplification products (Guo,

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1997). In RAPD each isolates had a unique and distinct fingerprinting patterns (Ramadass *et al*., 1997).

The aim of this study was detecting the polymorphism and the specific genomic variations among the different isolates of *C. pseudotuberculosis* isolated from infected sheep in Ras Sudr in South Sinai on the basis of their RAPD patterns by using RAPD-PCR technique.

Materials and Methods

Strains. Seven isolates of *C. pseudotuberculosis* isolated from infected sheep in Ras Sudr in South Sinai were used. These isolates were previously identified as *C. pseudotuberculosis* bacteriologically and molecularly by PCR (Photo1) (Goda *et al*., 2006).

Extraction of DNA. The DNA of 7 *C. pseudotuberculosis* isolates were extracted by cetyl trimethyl ammonium bromide (CTAB) according to (Sambrook *et al*., 1989).

Random amplified polymorphic DNA Fingerprinting (RAPD-PCR). It was performed according to (Pacheco *et al*., 1998). The total RAPD reaction volume was 50µl, containing 1µl (600 ng) of the extracted DNA from *C. pseudotuberculosis* isolates, 5µl 10 x PCR buffer, 2µl dNTPs (40 µM), 1µl (1.5 µ) DNA polymerase, 36 µl deionized distilled water and 1µl (50 pmol) of each of the following five primers of arbitrary sequence (MWG-Biotech AG). I-Sequence of primer 1 (D1247)II-Primer 2 (D1281) 5` AAG AGC CCG T 3, `5` AAC GCG CAA C3` III-Sequence of primer 3 (D1283) IV-Sequence of primer 4 (D1290) 5` GCG ATC CCC A 3`, 5` GTG GAT GCG A 3`V-Sequence of primer 5 (D14803) 5` AAA CGG TTG GGT GAG 3`. The mixture was overlaid with 40 µl paraffin oil and the reaction conditions were then placed into thermal cycler programmed as follows: Initial denaturation steps at 94°C for 4 min. followed by first cycle at 94°C for 45 seconds for denaturation, 37°C for 1 minute for annealing step and 72°C for 2 min. for extension step repeated for another 30 cycles. While the final extension were at 72°C for 10 min.

Agarose gel electrophoresis. The PCR products were visualized by agarose gel electrophoresis as described (Sambrook *et al*., (1989). 10µl PCR products were mixed with 2µl of 6x stock gel loading buffer (bromophenol blue 0.25%, xylene cyanol 0.25% and glycerol 30%) and then loaded onto a 1.5% agarose gel containing ethedium bromide at a concentration of 0.5 µl/ml. The gel was subjected to elcetrophoresis in 1X TAE for a suitable time that allow the bromophenol blue to run 2/3 of the gel length at 120 volts. 100 bp ladders (IBCO BRL, Life Technologies, Gent, Belgium) was inoculated in the gel as a molecular weight standard.

RAPD-PCR data analysis. The banding patterns generated by RAPD-PCR analysis were compared to determine the genetic relatedness of *C. pseudotuberculosis* isolates. The amplified fragments were scored either present (1) or absent (0). Bands of the same mobility were scored as identical. The similarity coefficient (f) between two isolates was defined using the formula of Nei and Li (1979). The genetic distance was carried out by Hillis and Morttiz (1990). A dendrogram was derived from the distance by the unweighed paired-group method, arithmetic mean (UPGMA) algorithm contained in the computer program MVSP (Multivariate statistical package) version 310 (1988-1999 Kovach Computer Services). The specific PCR and RAPD PCR were done in Biotech.lab Fac. Vet. Med. Cairo Univ., Giza, Egypt.

Results

In the present study 7 strains of *C. pseudotuberculosis* from the same origin were differentiated and characterized using DNA fingerprinting based on arbitrary random amplified polymorphic DNA polymerase chain reaction technique. The obtained unique patterns could be used as markers for the characterization of these strains and elucidate phylogenetic relationships among them (Fig.6). Different 5 random primers were tested with DNAs of the 7 strains of *C. pseudotuberculosis.* Table (1) revealed the results of primers that yield polymorphic electrophoretic banding patterns. Employing the synthesized 5 primers, the results revealed that the 5 primers yield numbers of polymorphic and share bands as in Table (1). Moreover, the number of bands with each individual primer were fallen in the range between 16 and 23 bands. The appearance of specific amplified bands from each strain was used as a measure of polymorphism at each locus and defined by an amplified band of characteristic size appear in 7 strains. The number of fragments generated by each primer in each strain was shown in Table (3). A total of 100 bands (Table 1) were detected in which 93

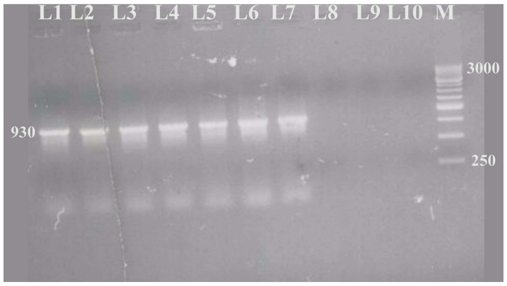


Photo (1): Agarose gel electrophoresis showing amplification of 930 bp fragment of *PLD* gene (specific) for *C. pseudotuberculosis*. Lanes 1, 2, 3, 4, 5, 6 and 7 showed positive amplifications. Meanwhile, lane 8, 9 and 10 showed negative results. Lane 11 (Marker), showed 250 bp ladder.

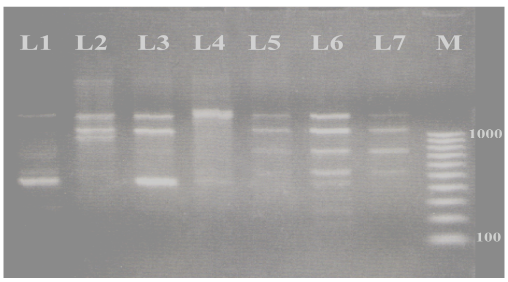


Photo (2): Agarose gel electrophoresis showing characteristic RAPD profile for 7 strains of *C. pseudotuberculosis* isolated from sheep using primer No. 1. L (Lane), M (Marker)

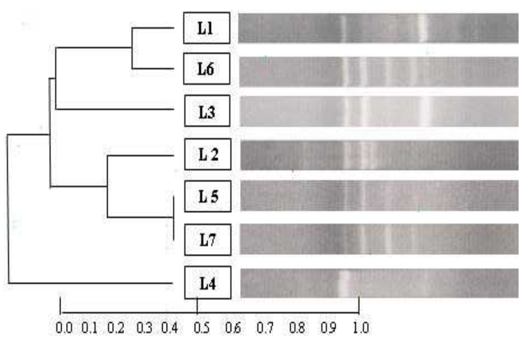


Fig (1): Phenogram demonstrating the relation among 7 strains of *C. pseudotuberculosis* based on a combined data set. (Primer No.1).

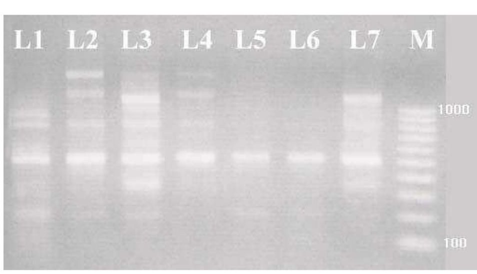


Photo (3): Agarose gel electrophoresis showing characteristic RAPD profile for 7 strains of *C. pseudotuberculosis* isolated from sheep using primer No.2. L (Lane), M (Marker).

were useful as RAPD-PCR markers generated by 5 random primers for the 7 strains of *C. pseudotuberculosis*. The size of the amplified fragment ranged from 122 bp for primer No.2 to 2237 bp for primer No.1. The least number of RAPD-PCR markers was detected for primer No.2 (2 markers out of 19 amplified bands), while the largest number of RAPD-PCR markers was detected for primer No.5 (9 markers out of 21 bands). However the shared band ranging from 1 band for primer No.1, 2 & 5 and 2 bands for primer No.3 and 4. The all 5 primers produce present unique band in strain No.1, 2, 3, 4, 6, and 7 as shown in Tables (1 , 2). These specific unique present bands considered as specific bands for each strain. The 11 out of 100 RAPD-PCR bands were found to be useful as strain-specific bands. The largest number of RAPD-PCR specific bands was scored for strain No.7 (3 markers), while (2 markers) were scored for strains No.3, 4 and 6 and 1 marker was record in each of strains No.1 and 2. In the meantime, the largest number of RAPD-PCR strain specific markers was generated by primer No.3 (4 markers) followed by each of primer No.1, 4 and 5 (2 markers). On the other hand, the least number of RAPD-PCR specific markers were generated by primer No.2 (1 marker). Also all 5 primers produced absent unique band which considered as specific absent band for each strain. The largest number of the RAPD-PCR absent specific markers was scored for strain No. 4 (3 markers) while (2 markers) were scored for strain No 7 and (1 marker) was recorded in strain 3 and 5.

The banding pattern of primer No.1 (Photo 2, Tables 4, 5), reactions showed differentiation between 5 strains which were found to be antigenically different. There was antigenic similarity between strain No.5 and 7. A total of 23 bands were characterized ranging between 168 and 2237 bp and the number of bands per lane ranged between 4 to 8 bands. All strains were found shared in 747 bp band. The similarity value ranged from 0.40 to 1.0 between the isolates of *C. pseudotuberculosis*.

With regard to primer No.2 (Photo 3 Tables 6, 7), 19 banding pattern were detected to be ranged from 122 to 1539 bp. The number of bands detected in each lane ranged from 2 to 8 bands. It differentiated between the 7 strains. It produced 1 shared band; 533 bp. The similarity value ranged from 0.28 to 0.857 between the isolates of *C. pseudotuberculosis*.

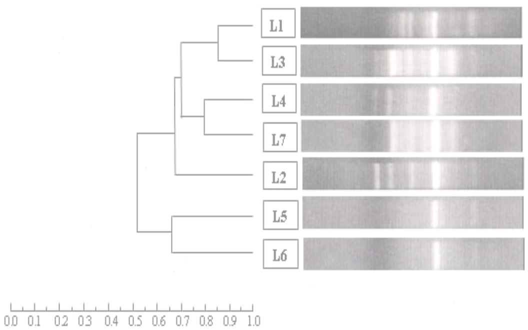


Fig (2): Phenogram demonstrating the relation among 7 strains of *C. pseudotuberculosis* based on a combined data set. (Primer No. 2).

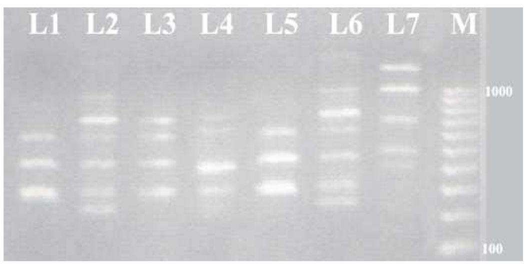


Photo (4): Agarose gel electrophoresis showing characteristic RAPD profile for 7 strains of *C. pseudotuberculosis* isolated from sheep using primer No.3.

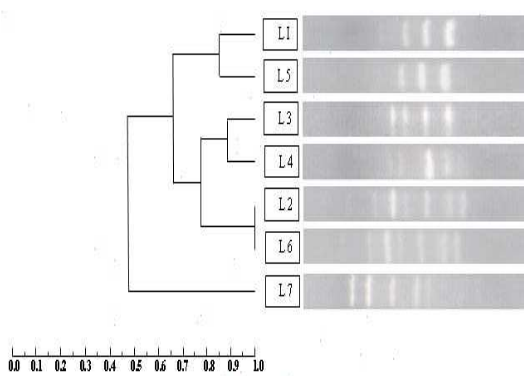


Fig (3): Phenogram demonstrating the relation among 7 strains of *C. pseudotuberculosis* based on a combined data set. (Primer No. 3).

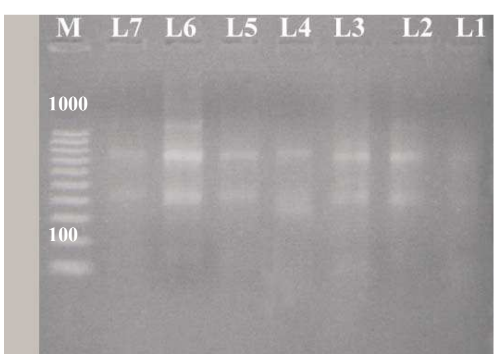


Photo (5): Agarose gel electrophoresis showing characteristic RAPD profile for the 7 strains of *C. pseudotuberculosis* isolated from sheep using primer No. 4.

With respect to primer No.3 Photo (4) and Tables (8 & 9), 21 banding pattern were detected to be ranged from 165 to 1458 bp. The count of bands per lane was ranging from 3 to 7 bands. It produces 2 shared bands at size 614 and 307 bp. This primer produced antigenic similarity between strains No.2 and 6 and produced antigenic difference between other strains. The similarity value ranged from 0.333 to 0.889 between the isolates of *C. pseudotuberculosis*.

Concerning primer No.4 (Photo 5, Tables 10, 11), 16 banding patterns were distinctly recognized ranging from 140 to 1097 bp. A range of 3 to 4 bands were detected in each lane. It produced 2 share bands in all investigated strains ranged at 743 bp and 432 bp. Primer 4 produced antigenic similarities between strains No.2 and 6 and also between strains No.1, 5 and 7. It differentiated between strains No. 3 and 4. The similarity value was ranged from 0.57 to 1.0 between the isolates of *C. pseudotuberculosis*.

Regarding primer No. 5 (Photo 6, Tables 12, 13), a total of 21 band patterns were characterized ranging from 220 to 1162 bp and the count of bands per lane was ranged from 4 to 9 bands. It produced one shared band in all the investigated strains at 510 bp. It differentiated between the 7 strains. The similarity value ranged from 0.133 to 0.545 between the isolates of *C. pseudotuberculosis*.

Phylogenetic relationships among the investigated *C. pseudotuberculosis* strains (no. = 7).

The 5 primers yield 100 reproducible RAPD band cluster analysis, only 1 dendrogram was obtained when 5 primers were included (Fig. 6, Table 14). Four clusters I, II, III and IV below similarity level 1.0. Cluster I included strains No.1, 3 and 5, cluster II include strains No.2 and 6, cluster III include strain No. 7 and cluster IV include strain No.4. In cluster I there was a similarity value 0.760 between strain No.1 and 3 and similarity value 0.638 between strain No.3 and 5. There was a similarity value 0.786 between strain No. 2 and 6 in cluster II. The dendrogram also revealed that strain No.1 and 4 were outliers with similarity value of 0.577.

This result revealed that, there was genetic difference between the isolates of *C. pseudotuberculosis* isolated from the infected sheep which were bred in the same origin and under the same conditions and the RAPD-PCR profiles obtained clearly distinguished between them.

Table (1): The outcome of polymorphic bands, share bands and specific present and absent bands in relation to type of the primer used for *C. pseudotuberculosis* identification.

Primer No.	Total No. of bands	No. of poly-morphic bands	No. of share bands	No. of specific present bands	No. of specific absent bands
1	23	22	1	2	3
2	19	18	1	1	1
3	21	19	2	4	1
4	16	14	2	2	1
5	21	20	1	2	1
Total	100	93	7	11	7

Table (2): Some specific present and absent unique bands used as a characteristic markers of a number of *C. pseudotuberculosis* strains obtained with all primers.

Primer code No.	Specific present bands		Specific absent bands	
	Strain No.	Band No.	Strain No.	Band No.
1	4	2	4	4 , 9 &
	6	20	3	12
2	6	18	5	6
3	1	6	7	10
	7	6, 14 & 20		
4	3	8	4	15
	4	12		
5	2	9	7	18
	3	7		

Table (3): The number of bands generated by 5 primers with each strains

Code No.	Strain No. 1	Strain No. 2	Strain No. 3	Strain No. 4	Strain No. 5	Strain No. 6	Strain No. 7
1	8	6	6	6	4	8	4
2	6	5	8	5	2	4	5
3	4	7	4	5	3	7	8
4	3	4	4	3	3	4	3
5	7	6	9	5	4	5	4
Total	28	28	31	24	16	28	24

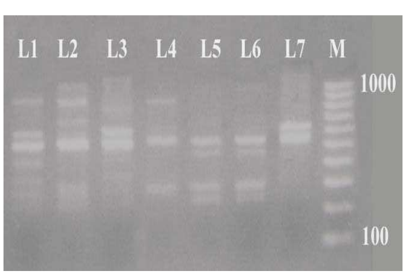


Photo (6): Agarose gel electrophoresis showing characteristic RAPD profile for the 7 strains of *C. pseudotuberculosis* isolated from sheep using primer No 5.

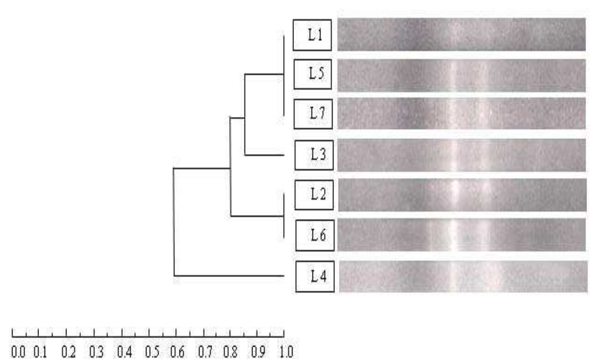


Fig (4): Phenogram demonstrating the relation among 7 strains of *C. pseudotuberculosis* based on a combined data set. (Primer No. 4)

Table (4): Scoring sheet and polymorphic RAPD marker obtained in RAPD reaction using primer No. 1 with 7 strains of *C. pseudotuberculosis*.

Bands	Mol.Wt	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Marker
1	2337.58	0	1	1	1	0	0	0	0
2	1892.00	0	0	0	1	0	0	0	0
3	1369.56	1	1	1	1	1	1	1	0
4	1071.65	1	1	1	0	1	1	1	0
5	1000.00	0	0	0	0	0	0	0	1
6	963.72	0	1	0	1	0	0	0	0
7	900.00	0	0	0	0	0	0	0	1
8	800.00	0	0	0	0	0	0	0	1
9	747.23	1	1	1	0	1	1	1	0
10	700.00	0	0	0	0	0	0	0	1
11	600.00	0	0	0	0	0	0	0	1
12	525.48	1	1	0	1	1	1	1	0
13	507.44	0	0	0	0	0	0	0	0
14	500.00	0	0	0	0	0	0	0	1
15	447.39	1	0	1	1	0	1	0	0
16	400.00	0	0	0	0	0	0	0	1
17	337.31	1	0	0	0	0	1	0	0
18	300.00	0	0	0	0	0	0	0	1
19	270.49	1	0	1	0	0	0	0	0
20	246.47	0	0	0	0	0	1	0	0
21	200.00	0	0	0	0	0	0	0	1
22	168.11	1	0	0	0	0	1	0	0
23	100.00	0	0	0	0	0	0	0	1
No. of Band.		8	6	6	6	4	8	4	10

Table (5): Similarity index between *C. pseudotuberculosis* amplified using primer No.1.

	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
Lane 1	1.000						
Lane 2	0.571	1.000					
Lane 3	0.714	0.667	1.000				
Lane 4	0.429	0.667	0.500	1.000			
Lane 5	0.667	0.800	0.600	0.400	1.000		
Lane 6	0.875	0.571	0.571	0.429	0.667	1.000	
Lane 7	0.667	0.800	0.600	0.400	1.000	0.667	1.000

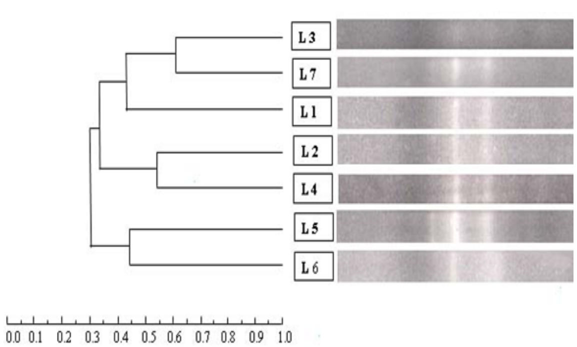


Fig (5): Phenogram demonstrating the relation among 7 strains of *C. pseudotuberculosis* based on a combined data set. (Primer No. 4)

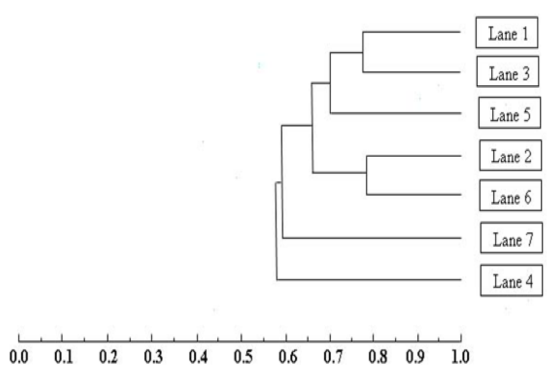


Fig (6): Dendrogram of DNAs using average linkage (between groups)

Table (6): Scoring sheet and polymorphic RAPD marker obtained in RAPD reaction using primer No. 2 with 7 strains of *C. pseudotuberculosis*.

Bands	Mol.Wt	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Marker
1	1539.24	0	1	1	1	0	0	0	0
2	1196.76	0	1	1	1	0	0	1	0
3	1000.00	0	0	0	0	0	0	0	1
4	993.69	1	0	1	0	0	0	0	0
5	900.00	0	0	0	0	0	0	0	1
6	849.36	1	1	1	1	0	1	1	0
7	800.00	0	0	0	0	0	0	0	1
8	700.00	0	0	0	0	0	0	0	1
9	639.38	1	0	1	0	0	0	1	0
10	600.00	0	0	0	0	0	0	0	1
11	533.30	1	1	1	1	1	1	1	0
12	500.00	0	0	0	0	0	0	0	1
13	400.00	0	0	0	0	0	0	0	1
14	362.32	1	0	1	1	0	0	1	0
15	300.00	0	0	0	0	0	0	0	1
16	218.37	1	1	1	0	1	1	0	0
17	200.00	0	0	0	0	0	0	0	1
18	122.19	0	0	0	0	0	1	0	0
19	100.00	0	0	0	0	0	0	0	1
No. of Band.		6	5	8	5	2	4	5	10

Table (7): Similarity index between *C. pseudotuberculosis* amplified using primer No. 2.

	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
Lane 1	1.000						
Lane 2	0.545	1.000					
Lane 3	0.857	0.769	1.000				
Lane 4	0.545	0.800	0.769	1.000			
Lane 5	0.500	0.571	0.400	0.286	1.000		
Lane 6	0.600	0.667	0.500	0.444	0.667	1.000	
Lane 7	0.727	0.600	0.769	0.800	0.286	0.444	1.000

Discussion

C. pseudotuberculosis is the causative agent of C. L in sheep and goats. This disease is characterized by the development of pyogranuloma in the lymph nodes and lung tissue (Pepin *et al*., 1997). Previous studies regarding characterization *C. pseudotuberculosis* have been hampered by the wide variation in the biochemical characteristics of the organisms (Collett *et al*., 1994). Some of these variations may be attributed to the different identification methods and technique, but there may also be individual strain to strain variation (ovine and caprine) (Kathleen *et al*., 2000). This was in agreement with the obtained results in that; there was great variation between different isolates of *C. pseudotuberculosis* isolated from sheep. The 7 isolates of *C. pseudotuberculosis* were differentiated by presence or absence of one or more bands at different position, this result was in agreement with Tenover *et al*., (1995), they differentiated between the *C. pseudotuberculosis* isolated from sheep, goats and horses by using Pulsed field gel electrophoresis (PFGE) by the presence or absence of one or more bands at various position. They also, recorded that, these varieties can be caused by a point mutation or by insertion or deletion, resulting in three or two fragments differences. In this study, the 5 chosen primers were able to differentiate between the 7 isolates of *C. pseudotuberculosis* according to band patterns, intensity, molecular weight of bands, presence or absence of one or more bands and similarity coefficient. This proved that, the RAPD-PCR is a powerful tool that produced unique, and discernible genomic fingerprints (Caetane-Anolles, *et al*., 1991; Woods *et al*., 1993 ; Fan *et al*., 1995). Hence, RAPD-PCR, has been used to study heterogenicity in closely related organisms as

Table (8): Scoring sheet and polymorphic RAPD marker obtained in RAPD reaction using primer No. 3 with 7 strains of *C. pseudotuberculosis*.

Bands	MolWt	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Marker
1	1458.11	0	1	0	0	0	1	0	0
2	1304.27	0	0	0	0	0	0	1	0
3	1050.00	0	1	0	0	0	1	1	0
4	1000.00	0	0	0	0	0	0	0	1
5	900.00	0	0	0	0	0	0	0	1
6	848.88	1	0	0	0	0	0	0	0
7	800.00	0	0	0	0	0	0	0	1
8	733.16	0	1	1	1	0	1	1	0
9	700.00	0	0	0	0	0	0	0	1
10	614.73	1	1	1	1	1	1	0	0
11	600.00	0	0	0	0	0	0	0	1
12	520.00	1	1	1	1	1	1	1	0
13	500.00	0	0	0	0	0	0	0	1
14	419.13	0	0	0	0	0	0	1	0
15	400.00	0	0	0	0	0	0	0	1
16	307.22	1	1	1	1	1	1	1	0
17	300.00	0	0	0	0	0	0	0	1
18	238.40	0	1	0	1	0	1	1	0
19	200.00	0	0	0	0	0	0	0	1
20	165.12	0	0	0	0	0	0	1	0
21	100.00	0	0	0	0	0	0	0	1
No. of Bands		4	7	4	5	3	7	8	10

Table (9): Similarity index between *C. pseudotuberculosis* **amplified using primer No. 3.**

	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
Lane 1	1.000						
Lane 2	0.545	1.000					
Lane 3	0.750	0.727	1.000				
Lane 4	0.667	0.833	0.889	1.000			
Lane 5	0.857	0.600	0.857	0.750	1.000		
Lane 6	0.545	1.000	0.727	0.833	0.600	1.000	
Lane 7	0.333	0.667	0.500	0.615	0.364	0.667	1.000

Table (10): Scoring sheet and polymorphic RAPD marker obtained in RAPD reaction using primer No. 4 with 7 strains of *C. pseudotuberculosis*.

Bands	Mol.Wt	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Marker
1	1097.86	0	1	0	0	0	1	0	0
2	1000.00	0	0	0	0	0	0	0	1
3	900.00	0	0	0	0	0	0	0	1
4	800.00	0	0	0	0	0	0	0	1
5	743.50	1	1	1	1	1	1	1	0
6	700.00	0	0	0	0	0	0	0	1
7	600.00	0	0	0	0	0	0	0	1
8	532.56	0	0	1	0	0	0	0	0
9	500.00	0	0	0	0	0	0	0	1
10	432.49	1	1	1	1	1	1	1	0
11	400.00	0	0	0	0	0	0	0	1
12	351.88	0	0	0	1	0	0	0	0
13	300.00	0	0	0	0	0	0	0	1
14	200.00	0	0	0	0	0	0	0	1
15	140.80	1	1	1	0	1	1	1	0
16	100.00	0	0	0	0	0	0	0	1
No. of Bands		3	4	4	3	3	4	3	10

Table (11): Similarity index between *C. pseudotuberculosis* amplified using primer No. 4.

	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
Lane 1	1.000						
Lane 2	0.857	1.000					
Lane 3	0.857	0.750	1.000				
Lane 4	0.667	0.571	0.571	1.000			
Lane 5	1.000	0.857	0.857	0.667	1.000		
Lane 6	0.857	1.000	0.750	0.571	0.857	1.000	
Lane 7	1.000	0.857	0.857	0.667	1.000	0.857	1.000

Table (12): Scoring sheet and polymorphic RAPD marker obtained in RAPD reaction using primer No. 5 with 7 strains of *C. pseudotuberculosis*.

Bands	Mol.Wt	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Marker
1	1161.79	0	0	1	0	0	0	1	0
2	1065.63	0	0	1	0	0	0	1	0
3	1000.00	0	0	0	0	0	0	0	1
4	974.55	0	1	0	1	0	1	0	0
5	900.00	0	0	0	0	0	0	0	1
6	800.00	1	1	0	1	0	0	0	1
7	766.01	0	0	1	0	0	0	0	0
8	700.00	0	0	0	0	0	0	0	1
9	654.87	0	1	0	0	0	0	0	0
10	588.40	1	0	1	1	0	0	1	0
11	600.00	0	0	0	0	0	0	0	1
12	510.54	1	1	1	1	1	1	1	0
13	500.00	0	0	0	0	0	0	0	1
14	423.19	1	0	1	0	1	1	0	0
15	400.00	0	0	0	0	0	0	0	1
16	318.21	1	0	1	0	0	0	0	0
17	300.00	0	0	0	0	0	0	0	1
18	274.68	1	1	1	1	1	1	0	0
19	220.41	1	1	1	0	1	1	0	0
20	200.00	0	0	0	0	0	0	0	1
21	100.00	0	0	0	0	0	0	0	1
No. of Bands		7	6	9	5	4	5	4	10

Table (13): Similarity index between *C. pseudotuberculosis* amplified using primer No. 5.

	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
Lane 1	1.000						
Lane 2	0.308	1.000					
Lane 3	0.500	0.133	1.000				
Lane 4	0.500	0.545	0.286	1.000			
Lane 5	0.364	0.200	0.308	0.222	1.000		
Lane 6	0.333	0.364	0.286	0.400	0.444	1.000	
Lane 7	0.364	0.200	0.615	0.444	0.250	0.222	1.000

Table (14): Records of similarity index of DNA (combined data).

	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7
Lane 1	1.000						
Lane 2	0.607	1.000					
Lane 3	0.780	0.644	1.000				
Lane 4	0.577	0.731	0.618	1.000			
Lane 5	0.727	0.682	0.638	0.500	1.000		
Lane 6	0.714	0.786	0.610	0.577	0.727	1.000	
Lane 7	0.577	0.615	0.655	0.583	0.550	0.577	1.000

this method detects difference in the DNA sequence at sites in the genome that are defined by the primers used . Sequence variation is revealed by the number and length of amplified products which may be phylogenetically conserved and this method is advantage for strains or isolates identification. Although in this study the isolation of *C. pseudotuberculosis* from infected sheep which were housed together and received the same hygienic measure, sanitary management, breeding and feeding programs, an unexpected finding was antigenic difference between the 7 isolates. This may be due to individual variation in the nucleotide sequences between the different isolates of the same origin. This fact proved that, RAPD-PCR can reproducibly distinguish even closely related strains of bacterial species, and this agree with (Geary *et al*., 1994), they stated that, RAPD-PCR result in strain specific arrays of DNA fragments can reproducibly distinguished closely related strains of bacterial species. In this study, the RAPD PCR genetically differentiated between different isolates of *C. pseudotuberculosis* isolated from sheep and each isolate has its own genetic marker, as the RAPD differentiated between different serovars of other bacterial microorganisms and this coincides with (Abbas *et al*. 2000; Goda, 2003; Moussa *et al*. 2006) who proved that RAPD markers as genetic markers were used to monitor genetic variability between *Pasteurella multocida* vaccinal strains. Also it can be used as a rapid method for genetic differentiation between *E. coli* of avian origin. Meanwhile, RAPD differentiated genetically between different isolates of *Salmonella* serovars of bird origin. Moreover RAPD PCR differentiated inter–and intra–serotype variation and epidemiology of different *E. coli* serotypes, rather than biochemical and serological identification. The result of this investigation revealed for the first time that, the 7 isolates of *C. pseudotuberculosis* which were isolated from the infected sheep were differed in their phylogenecity and phenogenecity and this may be due to the presence of novel serovars of this microorganism.

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الخصائص الجزيئية لعترات كورينى السل الكاذب المعزول من الأغنام باستخدام التمديد العشوائى لأجزاء الحمض النووي

تم تحديد الاختلافات الوراثية ودرجة القرابة بين عترات كورينى السل الكاذب المعزول من الأغنام والتى تم الكشف عنها باستخدام اختبار تفاعل البلمرة المتسلسل باستخدام خمسة بادئات عشوائية عن طريق التمديد العشوائي لأجزاء الحمض النووي وذلك بالفصل الكهربي فى جل الأجاروز. هذا وتكون الاختلافات فى مناظر الشرائط مميزة لكل عترة. وقد أوضحت النتائج اختلافات فى التركيب الجينى ودرجة القرابة بين العترات المعزولة. وربما يعزى ذلك إلى وجود عترات جديدة من ميكروب كورينى السل الكاذب.